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Persistent Organic Pollutants Modify Gut Microbiota–Host Metabolic Homeostasis in Mice Through Aryl Hydrocarbon Receptor Activation

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Materials and Methods

NMR-based metabolomics experiment

Sample preparation

^{1}H NMR Spectroscopy

Spectral data processing and multivariate data analysis

Figure S1. AHR-null liver extract reporter assay. AHR-responsiveness of extracts was examined using hepatoma reporter line, Hepa 1.1. Reporter cells were treated with 0.1 µL of control or TCDF liver extracts for 4 h. Data represent mean±S.E.M (n=5), T-test parameters: Unpaired, Two tailed, p-value < 0.001 (***).

Figure S2. (A) qPCR analysis of mRNA levels of bacterial *Firmicutes* and *Bacteroidetes* in the cecal content of vehicle and TCDF-treated $Ahr^{+/+}$ mice. (B-D) 16S rRNA gene sequencing analysis at the phylum and genus level of the cecal content. Data are presented as mean ± s. d, n = 6 and 5 per group for $Ahr^{+/+}$ and $Ahr^{-/-}$ mice, respectively; *p < 0.05, **p < 0.01, NS means no significance, two-tailed Student's t-test.

Figure S3. (A-D) Quantification of specific bile acids levels in liver and cecum of vehicle and TCDF-treated *Ahr*$^{+/+}$ mice (24 μg kg-1) by UPLC-TQMS. (E) qPCR analysis of mRNA levels of *Cyp7a1*, *Fxr* and *Shp* in the liver of vehicle and TCDF-treated *Ahr*$^{-/-}$ mice. Data are presented as mean ± s. d, n = 6 and 5 per group for *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ mice, respectively; *p < 0.05, **p < 0.01, NS, no significance, two-tailed Student's t-test. See also Table S1 and 2.

Figure S4. Western blot of Cyp7a1 and Actin levels in the liver.

Figure S5. Representative 600 MHz ^{1}H NMR spectra of liver (A and B), fecal (C and D) and cecal content (E and F) aqueous extracts from vehicle (B, D and F) and TCDF treated group (A, C and E). The regions of δ 6.1-9.20 and δ 0.6-3.1 in the liver spectra was vertically expanded 16 times and 4 times compared with the region of δ 3.1-4.7, respectively. The regions of δ 6.1-9.4 in the fecal aqueous extracts spectra were vertically expanded 16 times compared with the region of δ 0.5-4.5. The regions of δ 6.1-9.0 in the cecal content aqueous extracts spectra were vertically expanded 16 times compared with the region of δ 0.6-4.4. Keys: 1, lipid; 2, isoleucine; 3, leucine; 4, valine; 5, D-3-hydroxybutyrate; 6, lactate; 7, alanine; 8, acetate; 9, n-butyrate; 10, propionate; 11, threonine; 12, glutamate; 13, glutamine; 14, glutathione; 15, arginine; 16, proline; 17, creatine; 18, choline; 19, phosphorylcholine; 20, glycerophosphocholine; 21, β-glucose; 22, α-glucose; 23, unsaturated fatty acid; 24, TMAO; 25, tyrosine; 26, histidine; 27, phenylalanine; 28, formate; 29, betaine; 30, glycogen; 31, bile acid; 32, lysine; 33, N-acetyl aspartate; 34, oligosaccharides; 35, succinate; 36, taurine; 37, glycine; 38, inosine; 39, uridine; 40, fumarate; 41, nicotinurate; 42, adenosine; 43, uracil; 44, α-galactose; 45, α-arabinose; 46, α-xylose; 47, hypoxanthine; 48, glucose & amino acids; 49, ethanol; 50, pyruvate; 51,TMA; 52, raffinose; 53, stachyose; 54, methanol; 56, urocanate; 57, adenine; 58, α-ketoglutarate. See also Table S4.

Figure S6. O-PLS-DA scores (left) and coefficient-coded loadings plots for the models (right) from NMR spectra of aqueous duodenum (A), jejunum (B), ileum (C), and cecum (D) extracts from the vehicle and TCDF-treated *Ahr*$^{+/+}$ mice and fecal (E), cecal content (F) and liver (G) extracts from vehicle and TCDF-treated *Ahr*$^{-/-}$ mice.

Figure S7. Cross-validation with permutations test plots (200 permutations) for the PLS-DA models constructed from ^{1}H NMR data of liver (A, *Ahr*$^{+/+}$; B, *Ahr*$^{-/-}$), cecal content (C, *Ahr*$^{+/+}$; D, *Ahr*$^{-/-}$), fecal (E, *Ahr*$^{+/+}$; F, *Ahr*$^{-/-}$), duodenum (G), jejunum (H), ileum (I), and cecum (J) aqueous extracts from vehicle and TCDF-treated mice.

Figure S8. Two dimensional (2D) ^{1}H-^{1}H total correlation spectroscopy (TOCSY) for the identification of n-butyrate and propionate related to Figure 5A and B. The cross peaks of n-butyrate and propionate are highlighted with dotted and solid lines, respectively.

Figure S9. Measurements of n-butyrate and propionate concentration from NMR peaks integration relative to internal standard TSP in the cecal content (A) and fecal extracts (B) obtained from *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ vehicle and TCDF-treated mice. Data are presented as mean ± s.

d, n = 6 and 5 per group for $Ahr^{+/+}$ and $Ahr^{-/-}$ mice, respectively; ; *p < 0.05, **p < 0.01, NS, no significance, two-tailed Student's t-test.

Figure S10. qPCR analysis of mRNA levels of *Gpr41* and *Gpr43* expression in the colon (A) and *Gck*, *G6pase*, *Glut2* and *Pepck* expression in the liver of $Ahr^{+/+}$ vehicle and TCDF-treated $Ahr^{+/+}$ mice. Data are presented as mean ± s. d, n = 6 per group; *p < 0.05, two-tailed Student's *t*-test.

Table S1. Primer sequences for qRT-PCR, Related to the Experimental Procedures.

Table S2. Retention times and M/Z of bile acids in UPLC-TQD-MS measurements, Related to Figure 4.

Table S3. Significantly changed metabolites in the feces, cecal content, liver, and intestine of mice exposed to TCDF.

Table S4. ^{1}H NMR chemical shifts for metabolites assigned in liver, fecal and cecal content extracts.

Table S5. Cross-validation with permutation test and CV-ANOVA for PLS-DA and OPLS-DA models from NMR spectra of fecal, cecal content, liver and intestinal extracts.